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(71) Applicant (*for all designated States except US*): IDEC PHARMACEUTICALS CORPORATION [US/US]; 11011 Torreyana Road, San Diego, CA 92121 (US).

(72) Inventor; and
(75) Inventor/Applicant (*for US only*): COCCIA, Marco, A. [US/US]; 1059 Wilbur Avenue, San Diego, CA 92109 (US).

(74) Agents: GESS, E., Joseph et al.; Burns, Doane, Swecker, & Mathis, L.L.P., P.O. Box 1404, Alexandria, VA 22313-1404 (US).

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(54) Title: TUMOR ANTIGEN-SPECIFIC ANTIBODY-GP39 CHIMERIC PROTEIN CONSTRUCTS

(57) Abstract

This invention relates generally to the production of antigen-binding chimeric proteins also comprising an immunoeffector binding domain. More specifically, the invention relates to chimeric proteins comprising a tumor antigen-specific antibody or antibody fragment fused to the CD40 ligand, gp39. Compositions comprising, genetic constructions for, methods of use, and methods for producing the chimeric proteins are also disclosed.

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TUMOR ANTIGEN-SPECIFIC ANTIBODY-GP39 CHIMERIC PROTEIN CONSTRUCTS

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to novel chimeric proteins which comprise a tumor antigen-specific monoclonal antibody or some fragment thereof at the amino terminus fused to an immunostimulatory ligand at the carboxyl terminus. Such chimeric proteins serve to enhance anti-tumor immune responses at the site of the tumor by stimulating endogenous leukocytes which express receptor for the immunostimulatory ligand portion of the chimeric protein on their cell surface.

Technology Background

All publications and patent applications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

One important factor in the development of successful antitumor agents is the ability to design agents that will selectively kill tumor cells, while exerting relatively little, if any, harmful effects against normal tissues. This goal has been elusive to achieve, though, in that there are few qualitative differences between neoplastic and normal tissues. Because of this, much research over the years has focused on identifying tumor-specific "marker antigens" that can serve as immunological targets both for chemotherapy and diagnosis.

Many tumor-specific, or quasi-tumor-specific ("tumor-associated"), markers have been identified as tumor cell antigens that can be recognized by specific antibodies. Tumor-associated antigens (TAAs) are also expressed on normal cells to varying degrees, generally during different stages of

differentiation. Therefore, these antigens are also known as "differentiation antigens" (*Fundamental Immunology*, 3rd edition, W. Paul, ed., Raven Press, New York, 1993, Ch. 32). Despite the fact that TAAs are also expressed on normal cells to some extent, studies suggest that differences in expression levels between normal and malignant cells can often be enough to favor a therapeutic response (Oettgen, H.F. and Old, L.J., *The History of Cancer Immunotherapy*; DeVita et al. eds.; *Biologic Therapy of Cancer*, Lippincott, Philadelphia, 1991, pp. 87-119).

A variety of tumor-associated antigens (TAA) have been described as summarized in U.S. Patent No. 5,766,588, herein incorporated by reference in its entirety. In addition, assays for identifying tumor associated antigens and tumor specific antigens are known in the art, as described in U.S. Patent No. 5,763,164, also incorporated by reference in its entirety. Identification of tumor-specific and tumor-associated antigens will enable the identification and isolation of monoclonal antibodies specific for these antigens using well-established techniques in the art. However, it is generally the case that tumor specific antibodies will not in and of themselves exert sufficient antitumor effects to make them useful in cancer immunotherapy. Indeed, despite the variety of tumor-associated antigens which have now been identified, tumor cells remain poorly immunogenic.

Recently there has been a great deal of activity directed toward augmenting the immune response to tumor-associated antigens. These strategies attempt to alter the local immunological environment of the tumor cell so as to enhance the presentation of T-cell epitopes or to enhance the activation of tumor-specific T-lymphocytes (Pardoll, D., *Immunol. Today*, 14:310-316 [1993]). Such strategies have included: coinjection of tumor cells with adjuvants (Bartlett et al., 1972, *J. Natl. Cancer Inst.*, 48:245-257); "heterogenization" of tumor cells by infection

with viruses (Austin and Boone, 1979, *Adv. Cancer Res.*, 30: 301-345), by hapten conjugation (Mitchison, 1970, *Transplant Proc.*, 2: 92-103) or exposure to mutagens (Van Pel and Boon, 1982, *Proc. Natl. Acad. Sci USA*, 79: 4718-4722); transfection of tumor cells to express the B7 ligand to provide a costimulatory signal to T cells (Chen et al., 1992, *Cell*, 71: 1093-1102); and transfection of tumor cells to produce certain cytokines.

For instance, several cytokine genes have been introduced into tumor cells to produce vaccines having varying degrees of effect on both tumorigenicity and immunogenicity. Tumor cells have been modified with genes for interleukin-2 (IL-2) (Porgador, A., et al., *Int. J. Cancer*, 53:471-477 [1993]); interferon-alpha, (IFN-alpha)(Porgador, A., et al., *Int. Immunol.*, 150:1458-1570 [1993]); granulocyte-macrophage colony stimulating factor (GM-CSF) (Dranoff, G., et al., *Proc. Nat. Acad. Sci. USA*, 90:3539-3543 [1993]) and several others (see Paul's *Fundamental Immunology*, 3rd edition, p. 1158).

Attempts have also been made to modify expression of the MHC complex in order to improve the immunogenicity of tumor cells. Enhanced expression of MHC class I antigens following exposure of cells to cytokines or transfection of cells with genes specifying MHC class I antigens has been shown to render the treated cells more susceptible to lysis by CTLs (Weber, J. S., et al., *Cancer Res.*, 48:5818 [1988]); Zoller, M., *Int. J. Cancer*, 41:256 [1988]); Porgador, A., et al., *J. Immunogenet.*, 16:291 [1989]).

However, while the above approaches may have been shown to have some success in experimental models for some types of cancer, no general approach has been identified that enhances immune responses toward tumor cells in general. This may be due to the observation that, while most tumor cells can be shown to express some tumor-specific or tumor-associated antigens, the different

components of humoral and cell-mediated immunity react differently to and play different roles in the various types of cancer and tumor disease models (for a review, see Paul's *Fundamental Immunology*, 3rd ed., pp. 1158-1165).

For instance, some tumor cells are killed *in vitro* by a process involving antibody coating, opsonization and either phagocytosis by macrophages or antibody-dependent cell-mediated cytotoxicity in the presence of macrophages, natural killer cells or neutrophils. For some tumor cells, TNF produced by macrophages has been shown to be responsible for the cytotoxic effects observed *in vitro* (Urban et al. 1986, *Proc. Natl. Acad, Sci USA*, 83: 5233-5237). Corresponding effects *in vivo*, however, have not been readily achieved.

However, in rare instances, dramatic therapeutic effects have been achieved when cancer patients were treated with murine monoclonal antibodies (Houghton et al., 1985, *Proc. Natl. Acad. Sci. USA,* 82: 1242-1246; Goodman et al., 1990, *J. Clin. Oncol.,* 8: 1083). In addition, *in vivo* studies have suggested a role for NK cells in reducing metastatic dissemination of injected cancer cells (Talmadge et al., 1980, *J. Natl. Cancer Inst.,* 65: 801-809; Hanna et al., 1981, *J. Immunol.*, 127: 1754-1758). Moreover, there is some evidence that activation of macrophages *in vivo* plays a role in reducing metastasis in some experimental models (Whitworth et al., 1990, *Cancer Metastasis Review*, 4: 319-351).

For other types of tumors, i.e., virally- (LeClerc et al., 1973, *Int. J. Cancer,* 11: 426-432) and chemically-induced tumors (Rouse et al., 1972, *Nature New Biol.*, 238: 116-117), the requirement for T-cell mediated immunity has been clearly demonstrated. For example, in a model of MCA-induced tumors in mice, it was shown that transfer of immune cells but not of immune sera could transfer systemic tumor-specific immunity into irradiated mice (Old et a., 1962, *Ann. N.Y. Acad. Sci.*, 101: 80-106). Yet for other tumors, i.e., UV-induced tumors, CD8+

cytolytic T cells are required for tumor rejection. In fact, for these tumors, elimination of CD4+ T cells has even been shown to increase tumor rejection (Koeppen et al., 1993, *Transplant.*, 55).

Thus, it appears that different immune effector cells have varying degrees of relevance in the immune response to any particular type of tumor. For cellular cytolytic responses, analyses of effector cell phenotype, specificity and mechanism of action have shown that even a response against a single type of tumor cell can be multiclonal, mediated by multiple mechanisms, and/or directed against different determinants expressed on the same tumor cells (Anichini et al., 1987, *Immunol. Today*, 8: 385-389; North, 1984, *Contemp. Top. Immunobiol.*, 13: 243-257). Therefore, strategies which are aimed at enhancing systemic immunity via antigen-specific stimulation at the site of the tumor may be more universally applicable to a wider array of cancers than has been demonstrated for the various cancer therapeutic strategies reported thus far.

The immune system is capable of producing two types of antigen-specific responses to foreign antigens. Cell-mediated immunity is the term used to refer to effector functions of the immune system mediated by T lymphocytes. Humoral immunity is the term used to refer to production of antigen-specific antibodies by B lymphocytes. It has long been appreciated that the development of humoral immunity against most antigens requires not only antibody-producing B lymphocytes but also the involvement of helper T (hereinafter Th) lymphocytes. (Mitchison, *Eur. J. Immunol.*, 1:18-25 (1971); Claman and Chaperon, *Transplant Rev.*, 1:92-119 (1969); Katz et al., *Proc. Natl. Acad. Sci. USA*, 70:2624-2629 (1973); Raff et al., *Nature*, 226:1257-1260 (1970)).

While some B lymphocyte help is mediated by soluble molecules released by Th cells (for instance lymphokines such as IL-4 and IL-5), activation of B cells

also requires a contact-dependent interaction between B cells and Th cells. (Hirohata et al., *J. Immunol.*, 140:3736-3744 (1988); Bartlett et al., *J. Immunol.*, 143:1745-1765 (1989)). This indicates that B cell activation involves an obligatory interaction between cell surface molecules on B cells and Th cells. Such an interaction is further supported by the observation that isolated plasma membranes of activated T cells can provide helper functions necessary for B cell activation. (Brian, *Proc. Natl. Acad. Sci. USA*, 85:564-568 (1988); Hodgkin et al., *J. Immunol.*, 145:2025-2034 (1990); Noelle et al., *J. Immunol.*, 146:1118-1124 (1991)).

The process by which T cells help B cells to differentiate has been divided into two distinct phases; the inductive and effector phases (Vitetta et al., *Adv. Immunol.*, 45:1 (1989); Noelle et al., *Immunol. Today*, 11:361 (1990)). Although the inductive phase of T cell help is Ag-dependent and MHC-restricted (Janeway et al., *Immun. Rev.*, 101:34 (1988); Katz et al., *Proc. Natl. Acad. Sci., USA*, 10:2624 (1973); Zinkernagle, *Adv. Exp. Med. Biol.*, 66:527 (1976)); the effector phase of T cell helper function can be Ag-independent and MHC-nonrestricted (Clement et al., *J. Immunol.*, 132:740 (1984); Hirohata et al., *J. Immunol.*, 140:3736 (1988); Whalen et al., *J. Immunol.*, 143:1715 (1988)).

Although terminal B cell differentiation requires both contact- and lymphokine-mediated stimuli from T cells, intermediate stages of B cell differentiation can be induced by activated T cell surfaces in the absence of secreted factors (Crow et al., *J. Exp. Med.*, 164:1760 (1986); Brian, *Proc. Natl. Acad. Sci., USA*, 85:564 (1988); Sekita et al., *Eur. J. Immunol.*, 18:1405 (1988); Hodgkin et al., *J. Immunol.*, 145:2025 (1990); Noelle et al., *FASEB J,* 5:2770 (1991)). These intermediate effects on B cells include induction of surface CD23 expression (Crow et al., *Cell Immunol.*, 121:94 (1989)), enzymes associated with

cell cycle progression (Pollok et al., *J. Immunol.*, 146:1633 (1991)) and responsiveness to lymphokines (Noelle et al., *FASEB J*, 5:2770 (1989); Pollok et al., *J. Immunol.*, 146:1633 (1991)).

Recently some of the activation-induced T cell surface molecules and the corresponding ligands on the surface of B cells that are involved in B cell activation have been identified. A cell surface molecule, CD40, has been identified on immature and mature B lymphocytes which, when crosslinked by antibodies, induces B cell proliferation. Valle et al., *Eur. J. Immunol.*, 19:1463-1467 (1989); Gordon et al., *J. Immunol.*, 140:1425-1430 (1988); Gruder et al., *J. Immunol.*, 142:4144-4152 (1989).

CD40 has been molecularly cloned and characterized (Stamenkovic et al., *EMBO J.*, 8:1403-1410 (1989)). CD40 is expressed on B cells, interdigitating dendritic cells, macrophages, follicular dendritic cells, and thymic epithelium (Clark, *Tissue Antigens* 36:33 (1990); Alderson et al., *J. Exp. Med.*, 178:669 (1993); Galy et al., *J. Immunol.* 142:772 (1992)). Human CD40 is a type I membrane protein of 50 kDa and belongs to the nerve growth factor receptor family (Hollenbaugh et al., *Immunol. Rev.*, 138:23 (1994)). Signaling through CD40 in the presence of IL-10 induces IgA, IgM and IgG production, indicating that isotype switching is regulated through these interactions. The interaction between CD40 and its ligand results in a primed state of the B cell, rendering it receptive to subsequent signals.

Also, a ligand for CD40, gp39 (also called CD40 ligand or CD40L) has recently been molecularly cloned and characterized (Armitage et al., *Nature*, 357:80-82 (1992); Lederman et al., *J. Exp. Med.*, 175:1091-1101 (1992); Hollenbaugh et al., *EMBO J.*, 11:4313-4319 (1992)). The gp39 protein is expressed on activated, but not resting, $CD4^+$ Th cells. Spriggs et al., *J. Exp. Med.*,

176:1543-1550 (1992); Lane et al., *Eur. J. Immunol.*, 22:2573-2578 (1992); and Roy et al., *J. Immunol.*, 151:1-14 (1993). Cells transfected with gp39 gene and expressing the gp39 protein on their surface can trigger B cell proliferation and, together with other stimulatory signals, can induce antibody production. Armitage et al., *Nature*, 357:80-82 (1992); and Hollenbaugh et al., *EMBO J.*, 11:4313-4319 (1992). The gp39 ligand has been identified for the mouse (Noelle et al., *Proc. Natl. Acad. Sci. USA*, 89:6550 (1992); Armitage et al., *Nature*, 357:80 (1992)) and for humans (Hollenbaugh et al., *Embo. J.* 11:4313 (1992); Spriggs et al., *J. Exp. Met.*, 176:1543 (1992)). gp39 is a type II membrane protein and is part of a new gene super family which includes TNF-α, TNF-β and the ligands for FAS, CD27, CD30 and 4-1BB. $gp39^+$ T cells produce IL-2, IL-4 and IFN-γ (Van der Eetwegh et al., *J. Exp. Med.*, 178:1555 (1993)).

Unique insights into the novel role of gp39 in the regulation of humoral immunity have been provided by studies of a human disease, X-linked hyper-IgM syndrome (HIM). HIM is a profound, X-linked immunodeficiency typified by a loss in thymus dependent humoral immunity, the inability to produce IgG, IgA and IgE. Mutations in the gp39 gene were responsible for the expression of a non-functional gp39 protein and the inability of the helper T cells from HIM patients to activate B cells (Allen et al., *Science*, 259:990 (1993); Aruffo et al., *Cell*, 72:291 (1993); DiSanto et al., *Nature*, 361:541 (1993); Korthauer et al., *Nature*, 361:539 (1993)). These studies support the conclusion that early after T cell receptor engagement of the peptide/MHC class II complex, gp39 is induced on the cognate helper T cell, and the binding of gp39 to CD40 on the B cell induces the B cell to move into the cell cycle and differentiate to immunoglobulin (Ig) secretion and isotype switching.

Functional studies have shown that treatment of mice with anti-gp39 completely abolished the antibody response against thymus dependent antigens (SRBC and TNP-KLH), but not thymus independent antigens (TNP-Ficoll) (Foy et al., *J. Exp. Med.*, 178:1567 (1993)). In addition, anti-gp39 has been shown to prevent formation of memory B cells and germinal centers in mouse spleen (Foy et al., *J. Exp. Med.*, 180:157 (1994)). Moreover, CD40 ligation has also been shown to play an important role in IL2-R expression, IL-12 production and B7.1 expression by activated B cells (Nishioka and Lipsky, 1994, J. Immunol. 153: 1027). Collectively, these data provide extensive evidence that the interaction between gp39 on T cells and CD40 on B cells is essential for antibody responses against thymus dependent antigens.

CD40 ligation on dendritic cells (DC) stimulates IL-12 production (Koch et al., 1996, J. Exp. Med. 184(2):741-746) and enhances surface expression of ICAM-1 and B7.1 (Cella et al., 1996, J. Exp. Med. 184(2):747-752), all of which are important contributors to Th1-type, CTL-mediated immune responses (Heufler et al., 1996, *Eur. J. Immunol.*, 26:659). Thus, gp39-CD40 interaction appears to play a role in both cell-mediated immunity and antibody-specific immune responses, and is likely to enhance tumor specific responses in cancers which respond to either cell-mediated or humoral immune mechanisms.

The prediction that the gp39-CD40 interaction may be manipulated for therapeutic approaches to cancer is supported by two recent publications. First, Grossman et al. showed that transgenic expression of gp39 on neuroblastoma cells generated a significant reduction in tumor growth, even when only 1.5% of the tumor cells expressed gp39 ($p<0.001$) (*Human Gene Therapy*, 1997, 8(16): 1935-1943). In addition, the anti-tumor effects protected the mice from subsequent challenge by parental tumor cells, indicating that the responses were due to

systemic immunomodulation that once initiated, were gp39-independent. Second, Kato et al. showed that transfection of gp39 into chronic lymphocytic leukemia B cells induced autologous immune recognition and antileukemia immune response (*J. Clin. Invest.*, 1998, 101(5): 1144-1151).

However, one disadvantage in applying these reported strategies in the commercial setting, as well as in many of the strategies discussed above, is that cancer cells must be isolated from each individual patient and transfected with a gp39 construct in order to ensure proper cell compatibility in the subsequent immune response. Alternatively, one could attempt gene therapy techniques to target the gp39 construct to cancer cells, but such techniques have not been developed to the point where they are universally applicable to any type of cell on a predictable basis. In light of the forgoing, there remains a need for cancer therapeutic agents which enhance immune responses against targeted tumor-specific or tumor-associated antigens without the need for *ex vivo* cell modification or gene targeting techniques.

The present invention addresses the deficiencies of the prior art by providing chimeric protein constructs which contain a tumor antigen-specific antibody binding domain (or TAA-specific antibody) fused to the CD40 binding domain of the gp39 ligand. Such chimeric proteins provide effective and convenient therapeutic reagents which will enhance immune responses toward a wide variety of cancers for which a tumor-specific or tumor-associated antigen may be identified.

Chimeric proteins containing an antibody component fused to other types of molecules have previously been described and are known in the art. Antibody fusions have been generated to deliver cells, cytotoxins, or drugs to specific sites. An important use has been to deliver host cytotoxic cells, such as natural killer or

cytotoxic T cells, to specific cellular targets. (Staerz et al., *Nature*, 314:628 (1985); Songilvilai, et al., *Clin. Exp. Immunol.*, 79:315 (1990)). Another important use has been to deliver cytotoxic proteins to specific cellular targets. (Raso et al., *Cancer Res.*, 41:2073 (1981); Honda et al., *Cytotechnology*, 4:59 (1990)). A further important use has been to deliver anti-cancer non-protein drugs to specific cellular targets (Corvalan et al., *Intl. J. Cancer Suppl.*, 2:22 (1988); Pimm et al., *Brit. J. Can.*, 61:508 (1990)).

Antibodies have also been fused to toxic molecules for the purpose of delivering them specifically to cancer cells. For instance, U.S. Patent No. 5,756,699, herein incorporated by reference in its entirety, provides a thorough review of the state of art concerning immunotoxins, whereby the variable region of an antibody gene is fused to the gene for a bacterial toxin. It was hoped that such reagents could be used to target tumor cells, however, such fusion proteins have been shown to be immunogenic and toxic in animals.

Challida et al. disclose a B7.1-antibody fusion protein specific for the tumor-associated antigen HER2/*neu*. However, in contrast to the recombinant DNA constructs of the present invention, the construct disclosed in Challida et al. fuses the gene for the B7.1 ligand at the 5' end of the antibody nucleic acid such that the ligand is fused to the amino terminus of the antibody. This is an awkward position for the ligand portion of the chimeric protein as it has the potential to interfere with antigen binding by the antibody binding pocket. Moreover, the goal of the Challida reference was to stimulate T cells specifically, while the goal of the present invention is to provide an enhanced systemic immune response for the treatment of a wide variety of cancers.

U.S. Patent No. 5,767,260, also incorporated by reference herein in its entirety, discloses an immunoeffector-antibody fusion protein comprising a tumor

antigen-specific antibody fused to Phospholipase A activating protein ("PLAP"). PLAP is a protein that activates phospholipase A, a lipolytic enzyme which hydrolyzes the 2-acyl fatty acid ester of glycerophospholipids. This hydrolysis releases arachidonic acid which is converted into a number of biologically active compounds called eicosanoids. PLAP has been postulated to be involved in the inflammatory cascade in certain biological settings, and induces eicosanoid release and stimulation of joint inflammation, related to rheumatoid arthritis.

The ability of Fc receptors to trigger activation of phospholipase A_2 led to the finding that inhibitors of phospholipase A_2 could apparently inhibit phagocytosis by macrophages of IgG-coated particles (Lennartz et al., 1991, *J. Immunol.*, 147: 621-626). Conversely, the exogenous addition of arachidonic acid restores phagocytic capabilities. Thus, the goal of U.S. Patent No. 5,767,260 is to stimulate phagocytic activity at the region of tumors, in contrast to the present invention, which seeks to stimulate a systemic immune response.

In addition, the role of phospholipase A_2 in Fc receptor signaling is far from clear, and has been the subject of some debate due to studies which demonstrated that the block of arachidonic acid release in certain assay systems has not inhibited the phagocytosis of IgG-coated erythrocytes (Yamada et al., 1989, 142: 2457-2463). Moreover, even if PLAP-antibody fusion proteins are able to enhance phagocytosis at the site of some tumors, the extensive research summarized above indicates that phagocytosis alone will not be effective for all types of cancer.

Clearly, there remains a need in the art for therapeutic, immunomodulatory reagents which may be employed for the treatment of a wide variety of diseases by virtue of their ability to stimulate a systemic immune response.

SUMMARY OF THE INVENTION

The present invention pertains to dual function chimeric proteins comprising both an antigen binding domain and a receptor ligand binding domain, wherein said receptor ligand is involved in immune cell modulation and stimulation. Such proteins may be constructed by linking or bonding together two protein or protein fragments, or may be synthesized from recombinant DNA constructs as fusion proteins.

Preferably the chimeric proteins of the present invention are synthesized from recombinant DNA constructs. In general, such a construct comprises a nucleic acid encoding at least a heavy chain variable region binding domain of a disease antigen-specific antibody fused to a nucleic acid encoding at least a binding portion of an immunostimulatory ligand such that expression of said nucleic acid molecule yields a fusion protein having the heavy chain antibody variable region domain at its amino terminus and the binding portion of the immunostimulatory ligand at its carboxyl terminus. When expressed in a host cell with the corresponding light chain of the particular disease antigen-specific antibody, a chimeric protein is formed comprising at the very least an Fv fragment of the antibody fused to the binding domain of an immunostimulatory ligand.

A nucleic acid molecule according to the present invention may also comprise a nucleic acid encoding at least one antibody constant region. A construct with one heavy chain constant region will result in a fusion protein wherein the binding portion of the immunostimulatory ligand is fused to a Fab fragment of the disease antigen-specific antibody. A construct with at least part of the second constant region of the antibody will include sequences encoding the cysteine residue involved in heavy chain disulfide bridge formation, and will result in a fusion protein wherein the binding portion of the immunostimulatory ligand

is fused to an $F(ab')_2$ fragment of the disease antigen-specific antibody (See Figure 1). Fusion proteins comprising full length antibody proteins are also included.

A nucleic acid molecule according to the present invention may also comprise a nucleic acid encoding a light chain antibody or antibody fragment fused with said nucleic acid encoding said antibody variable region. In such a construct, the nucleic acid encoding said light chain antibody or antibody fragment is typically fused to said nucleic acid encoding said antibody variable region at the 5' end in such a way that upon expression, the light chain and heavy chain variable regions associate to form an antigen binding pocket (See Figure 1D). Proper association and folding of the two antibody fragments typically requires a flexible peptide linker, which is encoded by a nucleic acid present between the light chain and heavy chain coding regions in the nucleic acid construct.

The chimeric proteins of the present invention are specifically designed to enhance immune responses by cells of the immune system in the vicinity of diseased cells by providing a dual function binding protein that binds to both an antigen on the surface of the diseased cell and to a receptor on the surface of an immune cell. The diseased cell antigen may be a tumor antigen or a viral antigen, or any antigen that is specifically expressed on the particular cells targeted for enhanced immune responses.

The immune cell receptor may be any receptor specifically expressed on immune cells, but is preferably one which stimulates immune cell responses upon ligand interaction, such as cytokine production, costimulatory molecule expression, APC function or T helper cell stimulation. In particular, the immunostimulatory ligand is a CD40 ligand, and is most preferably gp39 or the receptor binding portion thereof.

The two binding portions of the dual function chimeric protein may optionally be connected by a linker peptide of variable length, which is encoded by an additional, in-frame coding region in the nucleic acid constructs of the present invention. The necessity of such a linker peptide will depend on the nature of the targeted antigen, i.e., its location and accessibility, the receptor for the immunostimulatory ligand, and the particular constraints of tertiary protein structure for each particular immunostimulatory ligand and antigen binding domain.

The present invention also encompasses chimeric proteins encoded by the nucleic acid constructs described above, as well as chimeric proteins that can be constructed by binding together the two separate protein domains, i.e., using bifunctional chelators or other linking proteins. Pharmaceutical compositions comprising the chimeric proteins of the present invention in a pharmaceutically acceptable carrier are also included. It has been demonstrated by Shopes (*J. Immunol.*, 148(9):2918-2922, 1992) that "tail-to-tail" dimeric IgG-IgG dimers having tetravalent binding could be generated through the formation of a disulfide linkage between individual heavy chains on the Ig molecule. A similar approach was used by Caron et al (*J. Exp. Med.*, 176:1191-1195, 1992). Both groups used a genetically engineered approach to artificially introduce a cysteine four amino acids from the carboxyl end of the heavy chain (position 444). Tetravalent IgG/IgG dimers were also developed using chemical approaches (Ghetie et al, *PNAS*, 94:7509-7514, 1997) in which a thioether linkage was generated between the IgG molecules. unfortunately, in the Ghetie approach, the chemical cross-linking was random and not limited to a specific site on the IgG heavy chain.

The present invention also includes vectors and host cells comprising the nucleic acid molecules described above. Host cells may be prokaryotic or

eukaryotic depending on the purpose for expressing the vector. For instance, prokaryotic cells will be more convenient for multiplying, isolating and maintaining vectors comprising the nucleic acid, whereas eukaryotic cells may be required for expression and isolation of properly folded and active protein depending on the antigen binding and ligand binding domains chosen for the nucleic acid construct. Host cells may also contain the nucleic acids of the present invention integrated into the chromosome. Any type of vector may be used depending upon the particular host cell chosen for expression of the nucleic acid, including prokaryotic and eukaryotic vectors, viral or phage vectors, etc.

Host cells which express the chimeric proteins of the present invention may be used in a method of making the chimeric proteins comprising expressing the nucleic acid molecule of the invention in a host cell and isolating the resulting chimeric protein. The particular method of protein purification will depend upon the particular expression system used as well as the nature of the chimeric protein. For instance, chimeric proteins having an antigen binding domain may be isolated and purified using columns made from the target antigen. Alternatively, purification columns may be designed using the immune cell receptor which interacts with the ligand binding domain. Classical methods of protein purification including DSFF or CM chromatography may also be used depending on the physical properties of the chimeric protein. Such techniques are well known in the art.

The chimeric proteins of the present invention may be used in a method of enhancing disease antigen-specific antibody responses in a subject who expresses the relevant disease antigen. Such a method comprises administering the chimeric protein of the invention to such a subject such that disease antigen-specific or systemic immune responses, i.e., cytokine production, costimulatory molecule

expression, APC function, helper and cytotoxic T cell stimulation, etc., are enhanced. Such enhanced immune response may also provide a method of treating a disease in a patient in need of such treatment, where such treatment may prevent, alleviate or cure the particular disease. Diseases which particularly benefit from the method of the present invention include cancer and viral infections such as AIDS.

It is anticipated that the present invention will have particular use in hospital or outpatient settings. Accordingly, kits comprising the chimeric proteins in sterile form whereby the proteins may be easily administrated to a patient are also encompassed in the present invention. For the laboratory setting where those of skill in the art might wish to purchase the vectors of the present invention for the purpose of modifying the vectors with alternative binding domains, kits comprising the nucleic acid molecules, vectors and appropriate host cells are also included. Columns for purifying the resulting chimeric protein may also be included.

Other aspects and variations of the present invention will become clear in the drawings and description to follow.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Diagrammatic representation of proposed chimeric TAA-specific mAb/gp39 immunotherapeutic fusion proteins and their corresponding DNAs.

Figure 2. A diagrammatic representation of the anti-tumor immune response generated by a TAA specific monoclonal antibody-gp39 chimeric protein construct.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. For purposes of the present invention, the following terms are defined.

A "nucleic acid molecule" may be DNA or RNA, and may contain modified nucleotide bases so long as such modified bases do not inhibit expression of the encoded chimeric protein.

A "heavy chain variable region binding domain" is the most minimal portion of an antibody heavy chain variable region capable of association with the corresponding light chain of the antibody and subsequent antibody recognition.

A "disease antigen" may be any antigen specifically expressed by, or shown to be preferentially associated with, a diseased cell. A "diseased cell" may be a tumor, cancer, or malignant cell, or a virally-infected cell. A tumor antigen may be tumor-specific, i.e., only expressed by tumor cells, or tumor-associated (TAA), i.e., also expressed by normal cells but perhaps at a different time or at a different level. A disease antigen for the purposes of the present invention must be expressed on the surface of a diseased cell, either by virtue of its own secretory signals, or in context with an MHC molecule. A disease antigen may also be expressed on the surface of a phagocytic cell, i.e., a macrophage, following phagocytosis, for instance in context with an MHC molecule.

An "antibody" according to the present invention may be from any species so long as it is specific for the targeted antigen. The antibody may be derived

from a humanized antibody or other antibody gene which has been genetically engineered. The "antibody" may be an antibody fragment, i.e., an Fv, FAB, F(ab') or $F(ab)_2$ fragment, the structure of all of which are known in the art as described in U.S. Patent No. 5,648,237, herein incorporated by reference.

When referring to nucleic acids, the term "fused" means that the two nucleic acids are fused in such a way that a single protein molecule or peptide chain is formed upon expression of the fused nucleic acid construct.

A "binding portion of an immunostimulatory ligand" means the minimal region of any ligand which interacts with or binds to the immunostimulatory receptor molecule which is targeted by the chimeric protein of the present invention. An "immunostimulatory receptor molecule" is any receptor on the surface of an immune cell, the ligation or binding of which stimulates an immune response from the cell on which it is expressed. For instance, when the targeted immunostimulatory receptor is CD40, the immunostimulatory ligand is any molecule which interacts with and binds to CD40, i.e., a CD40 monoclonal antibody or fragment thereof, gp39, or any other molecule which is demonstrated to bind specifically or particularly to CD40.

A "fusion protein" is a chimeric protein resulting from expression of a nucleic acid sequence which encodes peptide sequences derived or designed from more than one native protein sequence. The term "derived" indicates the sequences were designed from the native protein sequence, but may contain amino acid substitutions which do not inhibit, or perhaps even increase, the stability or functional capabilities of the chimeric protein. Such amino acid substitutions may be a necessary result of nucleotide base changes required for the formation of restriction endonuclease cleavage sites during construction of the recombinant DNA construct.

A nucleic acid encoding a "compatible" antibody light chain or light chain fragment contains a variable region domain which, upon expression, contributes to the formation of the antigen binding pocket. This nucleic acid molecule may be fused in frame to the N terminus of the heavy chain recombinant DNA construct by a sequence of nucleotides which encode a flexible peptide linker. This flexible peptide linker is designed with an appropriate length and sequence such that the light chain and heavy chain regions of the antibody may take on the native conformation of the antigen binding pocket.

A "subject who expresses a disease antigen" or a "patient" may be either human or animal. A patient may be "in need of treatment" without actually demonstrating physical symptoms of disease so long as the patient expresses the disease antigen on a diseased cell. The term "treatment" encompasses any therapeutic regimen where the aim is to prevent, alleviate or cure the particular disease, and may be accomplished if the disease progression is merely slowed or symptoms are alleviated for any period of time no matter how brief.

"Disease antigen-specific responses" may include cytokine production, costimulatory molecule expression, APC function, T helper cell stimulation, the infiltration of immune cells to the site of the diseased antigen or cell, such as, infiltration by T cells, B cells, macrophages and other lymphocytes. The immunostimulatory moieties in the fusion molecules of the invention can also cause or modulate, for example, the activation of lymphocyte cells, the expression of lymphocyte-specific compounds, the elaboration of antibodies, the enhancement of phagocytosis by phagocytes, and the enhancement of tumor cell lysis, or any immune cell response which is a specific result of the binding or ligation of the target immunostimulatory receptor.

The variable regions of both heavy and light chains show considerable variability in structure and amino acid composition from one antibody molecule to another, whereas the constant regions show little variability. The term "variable" as used in this specification refers to the diverse nature of the amino acid sequences of the antibody heavy and light chain variable regions. Each antibody recognizes and binds antigen through the binding site defined by the association of the heavy and light chain variable regions into an F[V]area. The light-chain variable region V[L]and the heavy-chain variable region V[H]of a particular antibody molecule have specific amino acid sequences that allow the antigen-binding site to assume a conformation that binds to the antigen epitope recognized by that particular antibody.

Within the variable regions are found regions in which the amino acid sequence is extremely variable from one antibody to another. Three of these so-called "hypervariable" regions or "complementarity-determining regions" (CDR's) are found in each of the light and heavy chains. The three CDR's from a light chain and the three CDR's from a corresponding heavy chain form the antigen-binding site.

The Chimeric Protein Construct

It is preferred that the chimeric proteins of the invention be constructed according to one of the following basic forms. The first form comprises an antigen binding, i.e., the variable region from an antibody heavy chain fused at its C terminus to an immunoeffector protein moiety. The protein may optionally contain a peptide spacer between the two functional domains, so that the structure will generally be: NH2-V[H]-immunoeffector moiety-COOH or NH2-V[H]-spacer-immunoeffector moiety-COOH. The construct may optionally contain the variable region from the corresponding light chain antibody fused to the N-

terminus, preferably by a flexible peptide linker, such that the basic form will generally be: NH2-V[L]-spacer-V[H]-immunoeffector moiety-COOH. The protein may also comprise constant region domains from both the light and heavy chains of the antibody, such that varying portions of a single chain antibody are fused to the N-terminus of the immunostimulatory ligand (See Figure 1).

Processes for creating single chain antibody fusions wherein a light chain variable region is fused to a heavy chain variable region are disclosed in U.S. Patent No. 5,767, 260, herein incorporated by reference. A computer-assisted method for designing a linker to bridge the variable domains is described more particularly in U.S. Pat. No. 4,704,692, also incorporated by reference in its entirety. A description of the theory and production of single-chain antigen-binding proteins is found in U.S. Pat. Nos. 4,946,778 and 5,260,203. Such single-chain antigen-binding proteins have been shown to have binding specificity and affinity substantially similar to that of the corresponding Fab fragment.

Linkers used in the fusion constructs of the invention can be any of those linkers known or used in the art. Skilled artisans will be able to determine the appropriate linker to be used for a particular construct. It is preferred that the linkers utilized in constructing the antigen-binding fusion proteins of the invention are between 0 and 50 amino acids in length.

In some cases it may be necessary to separate the antigen-binding part of a fusion protein from the immunoeffector part of the fusion protein by a peptide spacer, in order to preserve both activities of the fusion protein. It is also preferred that the spacers are between 0 and 50 amino acids in length.

U.S. Patent No. 5,648,237, herein incorporated by reference in its entirety, provides a thorough description of the various antibody fragments which result by including varying degrees of the antibody constant region. For instance, the term

Fv is defined to be a covalently or noncovalently-associated heavy and light chain heterodimer which does not contain constant domains. The term Fab' may be defined as a polypeptide comprising a heterodimer of the variable domain and the first constant domain of an antibody heavy chain, plus the variable domain and constant domain of an antibody light chain, plus at least one additional amino acid residue at the carboxyl terminus of the heavy chain C[H]1 domain including one or more cysteine residues. F(ab')2 antibody fragments are pairs of Fab' antibody fragments which are linked by a covalent bond(s). The Fab' heavy chain may include a hinge region. This may be any desired hinge amino acid sequence. Alternatively the hinge may be entirely omitted in favor of a single cysteine residue or, preferably a short (about 1-10 residues) cysteine-containing polypeptide. In certain applications, a common naturally occurring antibody hinge sequence (cysteine followed by two prolines and then another cysteine) is used; this sequence is found in the hinge of human IgG1 molecules (E. A. Kabat, et al., *Sequences of Proteins of Immunological Interest*, 3rd edition (National Institutes of Health, Bethesda, Md., 1987)). The hinge region may also be selected from another desired antibody class or isotype.

Depending on the animal used to develop antibodies toward the target disease antigen, the antibody portion of the chimeric protein may generate an anti-antibody response in the subject receiving the chimeric protein. To alleviate this immune response, antibodies may be "humanized" (i.e., if the subject is a human). A humanized antibody is generally understood to be an immunoglobulin amino acid sequence variant or fragment thereof which is capable of binding to a predetermined antigen and which comprises a FR (framework) region having substantially the amino acid sequence of a human immunoglobulin and a CDR

having substantially the amino acid sequence of a non-human immunoglobulin or a sequence engineered to bind to a preselected antigen.

Methods for identifying and immunizing with the target antigen and methods for the identification and isolation of monoclonal antibody-producing hybridomas specific for the target antigen are well-known techniques in the art, as is isolating cDNA fragments which encode the relevant light and heavy chain antibody fragments for further manipulation and cloning. A useful laboratory manual for such general techniques is Sambrook et al., 1989, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor New York, but a wide variety of other reference materials are readily accessible.

The chimeric protein constructs of the present invention comprise an immunostimulatory ligand binding domain. A preferred immunostimulatory ligand is gp39, which has been shown to bind to CD40 on the surface of antigen presenting cells and thereby enhance immune responses.

The chimeric proteins of the present invention may contain any portion of gp39 or other immunostimulatory ligand so long as that portion is capable of interacting with or binding to its cognate receptor, and the chimeric protein as a whole is able to effectuate an immune response stemming from ligation of this cognate receptor. Such binding portions may be identified using any assay known in the art for measuring the binding capabilities or affinities of peptide fragments of a ligand to the corresponding receptor. For instance, peptide fragments may be synthesized according to the known sequence of gp39, and used in a competitive binding assay with gp39+ T cells. Alternatively, peptide display libraries may be generated using techniques known in the art and screened for the capability to bind CD40+ cells. The entire extracellular portion of the gp39 molecule may also be used. In addition, the immunoeffector domain may lack ligand binding activity

individually so long as it is capable of assembly into a functional binding domain when expressed as a portion of the chimeric antigen-binding protein.

Vectors and Host Cells

The chimeric proteins of the present invention are preferably produced by recombinant DNA techniques. Thus, chimeric proteins are encoded by nucleic acid fusion constructs, which are cloned into expression vectors and expressed in appropriate host cells for the production of the chimeric protein. Such host cells may be prokaryotic or eukaryotic.

As reviewed in U.S. Patent No. 5,468,237, herein incorporated by reference in its entirety, many have had success with antibody expression and isolation using prokaryotic expression systems. In fact, U.S. Patent No. 5,648,237 discloses cloning vectors and methods for expressing antibodies and antibody fragments in *E. coli*, whereby the antibodies are secreted into and isolated from the periplasmic space of the microorganism. Also provided are methods for effecting covalent bond formation at the hinge region following antibody isolation.

The vector disclosed in U.S. Patent No. 5,648,237 is a dicistronic expression vector whereby the light chain and heavy chain fragments are each under the control of an inducible bacterial promoter. The redox environment in the bacterial periplasmic space apparently favors disulfide bond formation between light and heavy chains but not between the hinge cysteine residues. Others have had success in expressing heavy and light chains as a single polypeptide chain in bacteria. These disclosures are also incorporated by reference (Bird et al., 1988, *Science*, 242: 423-426; U.S. Patent Nos. 4,946,778 and 5,476,786; and Huston et al., 1988, *Proc. Natl. Acad. Sci. USA*, 1988, 85: 5879-5883).

Although U.S. Patent No. 5,648,237 indicates that Ig fusion proteins may be expressed in prokaryotic cells using the vectors described therein, depending on the immunoeffector moiety of the chimeric protein, i.e., the importance of glycosylation and proper protein secretion in maintaining binding function of the ligand, the chimeric proteins of the present invention may also be expressed using a eukaryotic, baculovirus or retrovirus expression system.

Vectors for designing recombinant antibody fusion proteins are well-known and available in the art. For instance, Challida-Eid et al. used an IgG3 expression vector to express their B7.1-IgG3 fusion protein (Coloma et al., 1992, Novel vectors for the expression of antibody molecules using variable regions generated by the polymerase chain reaction, *J. Immunol. Methods*, 152:89). Since the sequence of the Gp39 gene is known, one of ordinary skill in the art may readily amplify the required portion of the gene using PCR, while employing primers for such PCR which incorporate into the resulting nucleic acid convenient restriction sites for cloning. Such restriction sites depend on the sequence and length of the particular antibody used, and whether or not a linker region will also be incorporated.

Challida-Eid et al. expressed their B7.1-Ig fusion construct in non-secreting Sp2/0 cells. Since the chimeric proteins of the present invention contain an immunostimulatory ligand at the C-terminus, proteins may be purified by virtue of either the antigen binding domain or the immunoeffector portion. Techniques for preparing such columns and accomplishing the purification are also well-known in the art.

Administration of the Chimeric Proteins

The term "treatment" encompasses administration of compounds prophylactically to prevent or suppress an undesired condition, and therapeutic

administration to eliminate or reduce the extent or symptoms of the condition. Treatment according to the invention may be for a human or an animal so long as there are cells in said human or animal which express the target antigen. Treatment may be by systemic administration to the subject or by local application to an affected site.

The compositions of the present invention, i.e., compositions comprising a chimeric protein of the present invention, may be made into pharmaceutical compositions with appropriate pharmaceutically acceptable carriers or diluents. The chimeric proteins may be administered to a subject either singly or in combination with other chimeric proteins of the invention, or other known reagents commonly used in treatment regiments for the particular disease of interest. For instance, the proteins may also be used in therapy in conjunction with other anti-cancer or anti-viral drugs and biologicals, or in conjunction with other immune modulating therapy including bone marrow or lymphocyte transplants or medications.

As included within the scope of this invention, "acceptable" is defined as being compatible with other ingredients of the formulation and not injurious to the subject or other non-target cells. These carriers include those well known to practitioners in the art as suitable for oral, rectal, nasal, topical, buccal, sublingual, vaginal, or parenteral (including subcutaneous, intramuscular, intravenous, and intradermal) administration.

In the present case, it will be appreciated that the compounds according to the invention may also be used in the manufacture of pharmaceuticals for the treatment or prophylaxis of cancer and viral infections. Such formulations may be presented in unit-dose or multi-dose sealed containers, for example, ampules and vials, and may be stored in a freeze-dried (lyophilized) condition requiring

only the addition of the sterile liquid carrier, for example, water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

In the methods of the present invention, the mode of administration and dosage of protein chosen should be adequate to result in effective circulating levels of the chimeric protein. This will obviously depend on the type and stage of disease, location and number of diseased cells, level of antigen expression and type of antigen chosen (i.e., tumor-specific or tumor-associated), etc. However, dosages for intravenous administration will generally range from 0.001 mg/kg to about 10 mg/kg body weight of the patient, to be administered over several days or weeks by daily infusions depending on the patient's tolerance.

An "effective amount" of the composition is such as to produce the desired effect in a patient which can be monitored using several end-points known to those skilled in the art. For example, such effects could be monitored in terms of a therapeutic effect, e.g., alleviation of some symptom associated with the disease being treated, or further evidence suggesting enhanced immune response in the targeted area. These methods are by no means all-inclusive, and further methods to suit the specific application will be apparent to the ordinary skilled artisan.

WHAT IS CLAIMED:

1. A nucleic acid molecule comprising a nucleic acid encoding at least a heavy chain variable region binding domain of a disease antigen-specific antibody fused to a nucleic acid encoding at least a binding portion of an immunostimulatory ligand such that expression of said nucleic acid molecule yields a fusion protein having the antibody variable region domain at its amino terminus and the binding portion of the immunostimulatory ligand at its carboxyl terminus, wherein said immunostimulatory ligand is a CD40 ligand.

2. The nucleic acid molecule of Claim 1, further comprising a nucleic acid encoding at least one antibody constant region.

3. The nucleic acid molecule of Claim 1, further comprising a nucleic acid encoding a light chain antibody or antibody fragment fused with said nucleic acid encoding said antibody variable region.

4. The nucleic acid molecule of Claim 3, wherein said nucleic acid encoding said light chain antibody or antibody fragment is fused to said nucleic acid encoding said antibody variable region by a nucleic acid encoding a flexible peptide linker.

5. The nucleic acid molecule of Claim 1, wherein said disease antigen-specific antibody is specific for a tumor antigen.

6. The nucleic acid molecule of Claim 1, wherein said disease antigen-specific antibody is specific for a viral antigen.

7. The nucleic acid molecule of Claim 1, wherein said nucleic acid encoding said antibody variable region is fused to said nucleic acid encoding said binding portion of an immunostimulatory ligand by a nucleic acid encoding a linker peptide.

8. The nucleic acid of Claim 1, wherein said CD40 ligand is gp39.

9. A chimeric protein encoded by the nucleic acid molecule of Claim 1.

10. A chimeric protein comprising a variable region binding domain from a disease antigen-specific antibody at its amino terminus and an extracellular binding portion of an immunostimulatory ligand at its carboxyl terminus, wherein said immunostimulatory ligand is a CD40 ligand.

11. The chimeric protein of Claim 10, further comprising at least one antibody constant region.

12. The chimeric protein of Claim 10, further comprising a light chain antibody or antibody fragment.

13. The chimeric protein of Claim 12, wherein said light chain antibody or antibody fragment is fused to the amino terminal end of said protein by a flexible peptide linker.

14. The chimeric protein of Claim 10, wherein said disease antigen-specific antibody is specific for a tumor antigen.

15. The chimeric protein of Claim 10, wherein said disease antigen-specific antibody is specific for a viral antigen.

16. The chimeric protein of Claim 10, further comprising a linker peptide between said antibody variable region and said immunostimulatory ligand binding domain.

17. The chimeric protein of Claim 10, wherein said CD40 ligand is gp39.

18. A pharmaceutical composition comprising the chimeric protein of Claim 9.

19. A pharmaceutical composition comprising the chimeric protein of Claim 10.

20. A vector comprising the nucleic acid molecule of Claim 1.

21. A host cell comprising the nucleic acid molecule of Claim 1.

22. A method of making a chimeric protein comprising a variable region binding domain from a disease antigen-specific at its amino terminus and an extracellular binding portion of an immunostimulatory ligand at its carboxyl terminus comprising expressing the nucleic acid molecule of Claim 1 in a host cell and isolating the resulting chimeric protein.

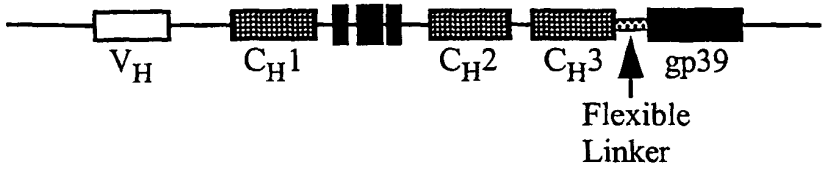
23. A method of enhancing disease antigen-specific antibody responses in a subject who expresses said disease antigen comprising administering the chimeric protein of Claim 10, to said subject such that disease antigen-specific immune responses are enhanced.

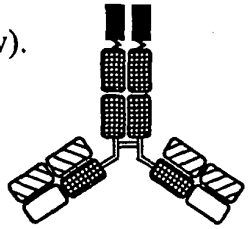
24. A method of treating a disease in a patient in need of such treatment comprising administering the chimeric protein of Claim 10.

25. A kit comprising the nucleic acid molecule of Claim 1.

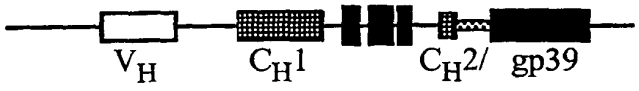
26. A kit comprising the chimeric protein of Claim 10.

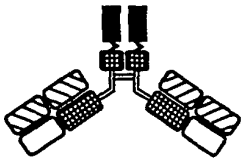
Construct A (only the relevant heavy chain sequences shown below).





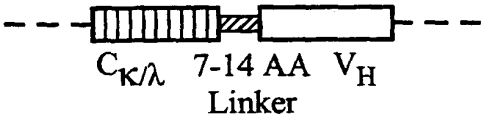
Construct B ($F(ab')_2$; mAb variable chain sequences not shown).





Construct C (Fab; mAb variable chain sequences not shown).

Construct D (Fab w/$C_{K/\lambda}$/V_H linked; mAb heavy chain constant domain, mAb light chain variable domain and gp39 sequences not shown).



Construct E (Fv).

Fig. 1

Proposed TAA-specific mAb/gp39 immunotherapeutic fusion proteins

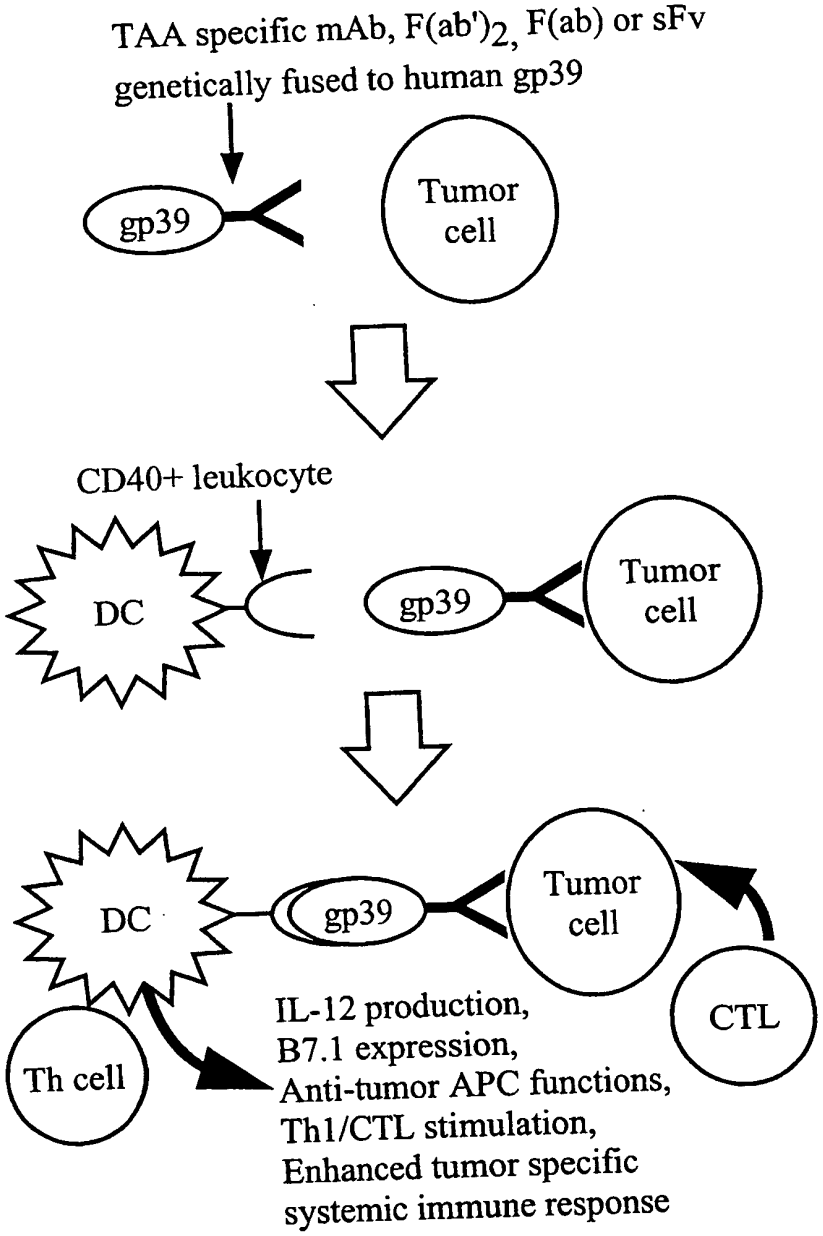


Fig. 2

Anti-tumor immune response induction by a TAA specific mAb-gp39 chimeric protein

TENT COOPERATION TRE[redacted]Y

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION CONCERNING SUBMISSION OR TRANSMITTAL OF PRIORITY DOCUMENT

(PCT Administrative Instructions, Section 411)

To:

GESS, E. Joseph /RLT
Burns, Doane, Swecker, & Mathis,
L.L.P.
P.O. Box 1404
Alexandria, VA 22313-1404
ÉTATS-UNIS D'AMÉRIQUE

Date of mailing (day/month/year) 03 February 2000 (03.02.00)	
Applicant's or agent's file reference 012712-810	**IMPORTANT NOTIFICATION**
International application No. PCT/US99/27654	International filing date (day/month/year) 23 November 1999 (23.11.99)
International publication date (day/month/year) Not yet published	Priority date (day/month/year) 23 November 1998 (23.11.98)
Applicant IDEC PHARMACEUTICALS CORPORATION et al	

1. The applicant is hereby notified of the date of receipt (except where the letters "NR" appear in the right-hand column) by the International Bureau of the priority document(s) relating to the earlier application(s) indicated below. Unless otherwise indicated by an asterisk appearing next to a date of receipt, or by the letters "NR", in the right-hand column, the priority document concerned was submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b).

2. This updates and replaces any previously issued notification concerning submission or transmittal of priority documents.

3. An **asterisk(*)** appearing next to a date of receipt, in the right-hand column, denotes a priority document submitted or transmitted to the International Bureau but not in compliance with Rule 17.1(a) or (b). In such a case, **the attention of the applicant is directed** to Rule 17.1(c) which provides that no designated Office may disregard the priority claim concerned before giving the applicant an opportunity, upon entry into the national phase, to furnish the priority document within a time limit which is reasonable under the circumstances.

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Priority date	Priority application No.	Country or regional Office or PCT receiving Office	Date of receipt of priority document
23 Nove 1998 (23.11.98)	60/109,607	US	24 Janu 2000 (24.01.00)

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Taïeb Akremi
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ENT COOPERATION TREA

SHAW PITMAN
SEP 27 2000

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: ROBIN L. TESKIN
BURNS, DOANE, SWECKER & MATHIS, LLP
P.O. BOX 1404
ALEXANDRIA, VA 22313-1404
UNITED STATES OF AMERICA

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	18 SEP 2000

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
012712-810	

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US99/27654	23 NOVEMBER 1999	23 NOVEMBER 1998

Applicant
IDEC PHARMACEUTICALS CORPORATION

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

BURNS, DOANE, SWECKER & MATHIS, L.L.P.
RECEIVED
SEP 2 1 2000
DOCKETED

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	PHILLIP CAMPBELL
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/416 (July 1992)*

ENT COOPERATION TREA

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: ROBIN L. TESKIN
BURNS, DOANE, SWECKER & MATHIS, LLP
P.O. BOX 1404
ALEXANDRIA, VA 22313-1404
UNITED STATES OF AMERICA

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	18 SEP 2000

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
012712-810	

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US99/27654	23 NOVEMBER 1999	23 NOVEMBER 1998

Applicant

IDEC PHARMACEUTICALS CORPORATION

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	PHILLIP CAMPBELL
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/IPEA/416 (July 1992)★

ENT COOPERATION TREA

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 012712-810	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US99/27654	International filing date *(day/month/year)* 23 NOVEMBER 1999	Priority date *(day/month/year)* 23 NOVEMBER 1998
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant **IDEC PHARMACEUTICALS CORPORATION**		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05 JUNE 2000	13 JULY 2000
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PHILLIP GAMBEL Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998)★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/27654

I. Basis of the report

1. With regard to the elements of the international application:*

☐ the international application as originally filed

☒ the description:
pages 1-28 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

☒ the claims:
pages 29-31 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

☒ the drawings:
pages 1-2 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

☒ the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

☐ the language of publication of the international application (under Rule 48.3(b)).

☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

☐ contained in the international application in printed form.

☐ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. ☒ The amendments have resulted in the cancellation of:

☒ the description, pages NONE

☒ the claims, Nos. NONE

☒ the drawings, sheets/~~fig~~ NONE

5. ☐ This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/27654

V. Reasoned statement under Article 35(2) with regard t novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	_____	YES
	Claims	_____	NO
Inventive Step (IS)	Claims	_____	YES
	Claims	_____	NO
Industrial Applicability (IA)	Claims	_____	YES
	Claims	_____	NO

2. citations and explanations (Rule 70.7)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:

The International Patent Classification (IPC) and/or the National classification are as listed below:

IPC(7): A61K/39/395; C07K/16/00, 16/18, 16/28, 16/46; C12N 15/12, 15/13 and US Cl.: 424/130.1, 133.1, 134.1, 141.1, 143.1, 152.1, 178.1, 192.1, 193.1; 530/387.1, 387.3, 388.1, 388.2, 388.22, 391.1; 536/23.1, 23.4, 23.5, 23.53

PCT REQUEST 012712-810

Original (for **SUBMISSION**) - printed on 23.11.1999 11:03:54 AM

0	**For receiving Office use only**	
0-1	International Application No.	
0-2	International Filing Date	
0-3	Name of receiving Office and "PCT International Application"	
0-4	**Form - PCT/RO/101 PCT Request**	
0-4-1	Prepared using	PCT-EASY Version 2.90 (updated 15.10.1999)
0-5	**Petition** The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty	
0-6	**Receiving Office (specified by the applicant)**	United States Patent and Trademark Office (USPTO) (RO/US)
0-7	**Applicant's or agent's file reference**	012712-810
I	**Title of invention**	TUMOR ANTIGEN-SPECIFIC ANTIBODY-GP39 CHIMERIC PROTEIN CONSTRUCTS
II	**Applicant**	
II-1	This person is:	applicant only
II-2	Applicant for	all designated States except US
II-4	Name	IDEC PHARMACEUTICALS CORPORATION
II-5	Address:	11011 Torreyana Road San Diego, CA 92121 United States of America
II-6	State of nationality	US
II-7	State of residence	US
II-8	Telephone No.	(858) 431-8500
II-9	Facsimile No.	(858) 431-8750
III-1	**Applicant and/or inventor**	
III-1-1	This person is:	applicant and inventor
III-1-2	Applicant for	US only
III-1-4	Name (LAST, First)	COCCIA, Marco, A.
III-1-5	Address:	1059 Wilbur Avenue San Diego, CA 92109 United States of America
III-1-6	State of nationality	US
III-1-7	State of residence	US

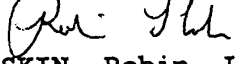
PCT REQUEST 012712-810

Original (for **SUBMISSION**) - printed on 23.11.1999 11:03:54 AM

IV-1	**Agent or common representative; or *address for correspondence*** The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as:	agent
IV-1-1	Name (LAST, First)	GESS, E. Joseph
IV-1-2	Address:	BURNS, DOANE, SWECKER, & MATHIS, L.L.P. P.O. Box 1404 Alexandria, VA 22313-1404 United States of America
IV-1-3	Telephone No.	(703) 836-6620
IV-1-4	Facsimile No.	(703) 836-2021
IV-2	**Additional agent(s)**	additional agent(s) with same address as first named agent
IV-2-1	Name(s)	TESKIN, Robin, L.
V	**Designation of States**	
V-1	Regional Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AP: GH GM KE LS MW SD SL SZ TZ UG ZW and any other State which is a Contracting State of the Harare Protocol and of the PCT EA: AM AZ BY KG KZ MD RU TJ TM and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT EP: AT BE CH&LI CY DE DK ES FI FR GB GR IE IT LU MC NL PT SE and any other State which is a Contracting State of the European Patent Convention and of the PCT OA: BF BJ CF CG CI CM GA GN GW ML MR NE SN TD TG and any other State which is a member State of OAPI and a Contracting State of the PCT
V-2	National Patent (other kinds of protection or treatment, if any, are specified between parentheses after the designation(s) concerned)	AE AL AM AT AU AZ BA BB BG BR BY CA CH&LI CN CR CU CZ DE DK DM EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

PCT REQUEST 012712-810

Original (for **SUBMISSION**) - printed on 23.11.1999 11:03:54 AM

V-5	**Precautionary D signation Statement** In addition to the designations made under items V-1, V-2 and V-3, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except any designation(s) of the State(s) indicated under item V-6 below. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.		
V-6	**Exclusion(s) from precautionary designations**	NONE	
VI-1	**Priority claim of earlier national application**		
VI-1-1	Filing date	23 November 1998 (23.11.1998)	
VI-1-2	Number	60/109,607	
VI-1-3	Country	US	
VI-2	**Priority document request** The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s):	VI-1	
VII-1	**International Searching Authority Chosen**	United States Patent and Trademark Office (USPTO) (ISA/US)	
VIII	**Check list**	number of sheets	electronic file(s) attached
VIII-1	Request	4	-
VIII-2	Description	28	-
VIII-3	Claims	3	-
VIII-4	Abstract	1	abstract.txt
VIII-5	Drawings	2	-
VIII-7	TOTAL	38	
	Accompanying items	paper document(s) attached	electronic file(s) attached
VIII-8	Fee calculation sheet	✓	-
VIII-10	Copy of general power of attorney	reference no. 012712	-
VIII-16	PCT-EASY diskette	-	diskette
VIII-17	Other (specified):	Receipt Postcard, Transmittal Letter, Check	-
VIII-18	**Figure of the drawings which should accompany the abstract**		
VIII-19	**Language of filing of the international application**	English	
IX-1	**Signature of applicant or agent**		
IX-1-1	Name (LAST, First)	TESKIN, Robin, L.	

FOR RECEIVING OFFICE USE ONLY

10-1	**Date of actual receipt of the purported international application**	

012712-810

PCT REQUEST

Original (for SUBMISSION) - printed on 23.11.1999 11:03:54 AM

10-2 10-2-1 10-2-2	Drawings: Received Not received	
10-3	Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application	
10-4	Date of timely receipt of the required corrections under PCT Article 11(2)	
10-5	International Searching Authority	ISA/US
10-6	Transmittal of search copy delayed until search fee is paid	

FOR INTERNATIONAL BUREAU USE ONLY

11-1	Date of receipt of the record copy by the International Bureau	

PCT (ANNEX - FEE CALCULATION SHEET)

012712-810

Original (for **SUBMISSION**) - printed on 23.11.1999 11:03:54 AM

(This sheet is not part of and does not count as a sheet of the international application)

0	**For receiving Office use only**				
0-1	International Application No.				
0-2	Date stamp of the receiving Office				
0-4	**Form - PCT/RO/101 (Annex) PCT Fee Calculation Sheet**				
0-4-1	Prepared using	PCT-EASY Version 2.90 (updated 15.10.1999)			
0-9	**Applicant's or agent's file reference**	012712-810			
2	**Applicant**	IDEC PHARMACEUTICALS CORPORATION, et al.			
12	**Calculation of prescribed fees**		fee amount/multiplier	total amounts (USD)	
12-1	Transmittal fee	T	⇨	240	
12-2	Search fee	S	⇨	700	
12-3	International fee Basic fee (first 30 sheets)	b1	455		
12-4	Remaining sheets		8		
12-5	Additional amount	(X)	10		
12-6	Total additional amount	b2	80		
12-7	b1 + b2 =	B	535		
12-8	Designation fees Number of designations contained in international application		83		
12-9	Number of designation fees payable (maximum 10)		10		
12-10	Amount of designation fee	(X)	105		
12-11	Total designation fees	D	1,050		
12-12	PCT-EASY fee reduction	R	-140		
12-13	Total International fee (B+D-R)	I	⇨	1,445	
12-14	Fee for priority document Number of priority documents requested		1		
12-15	Fee per document	(X)	15		
12-16	Total priority document fee	P	⇨	15	
12-17	**TOTAL FEES PAYABLE (T+S+I+P)**		⇨	2,400	
12-19	**Mode of payment**	cheque			
12-20	**Deposit account instructions** The receiving Office:	United States Patent and Trademark Office (USPTO) (RO/US)			
12-20-2	is hereby authorized to charge any deficiency or credit any over-payment in the total fees indicated above to my deposit account	✓			
12-21	Deposit account No.	02-4800			
12-22	Date	23 November 1999 (23.11.1999)			

PCT (ANNEX - FEE CALCULATION SHEET) 012712-810

Original (for **SUBMISSION**) - printed on 23.11.1999 11:03:54 AM

12-23	Name and signature	TESKIN, Robin, L. Robin J Teskin

VALIDATION LOG AND REMARKS

13-2-3	Validation messages Names	Green? Agent 1.: Where several first/given names are indicated, they should preferably be separated by a comma. Please verify.
13-2-6	Validation messages Contents	Green? Figure of the drawings which should accompany the abstract not specified. Please verify.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/27654

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K/39/395; C07K/16/00, 16/18, 16/28, 16/46; C12N 15/12, 15/13

US CL :Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG, BIOSIS, CA, EMBASE, MEDLINE, USPAT
search terms: cd40 ligand, cd40L, fusion protein, antibod?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,747,024 A (GRABSTEIN ET AL.) 05 May 1998, see entire document.	1-26
Y	US 5,767,260 A (WHITLOW ET AL.) 16 June 1998, see entire document.	1-26
Y	WO 97/29781 A1 (IMMUNEX CORPORATION) 21 August 1997, see entire document.	1-26

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 10 FEBRUARY 2000	Date of mailing of the international search report 08 MAR 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PHILLIP GAMBEL Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/27654

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/130.1, 133.1, 134.1, 141.1, 143.1, 152.1, 178.1, 192.1, 193.1; 530/387.1, 387.3, 388.1, 388.2, 388.22, 391.1; 536/23.1, 23.4, 23.5, 23.53

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

424/130.1, 133.1, 134.1, 141.1, 143.1, 152.1, 178.1, 192.1, 193.1; 530/387.1, 387.3, 388.1, 388.2, 388.22, 391.1; 536/23.1, 23.4, 23.5, 23.53

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 012712-810	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/US99/27654	International filing date *(day/month/year)* 23 NOVEMBER 1999	(Earliest) Priority Date *(day/month/year)* 23 NOVEMBER 1998
Applicant IDEC PHARMACEUTICALS CORPORATION		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (See Box I).

2. [ ] **Unity of invention is lacking** (See Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] transcribed by this Authority.

4. With regard to the **title**,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,
   - [X] the text is approved as submitted by the applicant.
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/27654

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :A61K/39/395; C07K/16/00, 16/18, 16/28, 16/46; C12N 15/12, 15/13
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG, BIOSIS, CA, EMBASE, MEDLINE, USPAT
search terms: cd40 ligand, cd40L, fusion protein, antibod?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,747,024 A (GRABSTEIN ET AL.) 05 May 1998, see entire document.	1-26
Y	US 5,767,260 A (WHITLOW ET AL.) 16 June 1998, see entire document.	1-26
Y	WO 97/29781 A1 (IMMUNEX CORPORATION) 21 August 1997, see entire document.	1-26

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 10 FEBRUARY 2000	Date of mailing of the international search report 07 MAR 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PHILLIP GAMBEL Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/27654

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/130.1, 133.1, 134.1, 141.1, 143.1, 152.1, 178.1, 192.1, 193.1; 530/387.1, 387.3, 388.1, 388.2, 388.22, 391.1; 536/23.1, 23.4, 23.5, 23.53

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

424/130.1, 133.1, 134.1, 141.1, 143.1, 152.1, 178.1, 192.1, 193.1; 530/387.1, 387.3, 388.1, 388.2, 388.22, 391.1; 536/23.1, 23.4, 23.5, 23.53